Strain of passive elements during force enhancement by stretch in frog muscle fibres

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1. The force enhancement during and after stretch (0·15 μm per sarcomere) was studied during fused tetani of single fibres isolated from the anterior tibialis muscle of *Rana temporaria* (0·5–3·6 °C; sarcomere length, 2·05–2·65 μm). Changes in length were recorded simultaneously from the fibre as a whole (puller movement) and from marked segments (~0·5 mm in length) of the same fibre.

2. The residual force enhancement after stretch (recorded at the end of a long tetanus) was found to be linearly related to the slow component of tension rise during the stretch ramp.

3. The fibres were released to shorten against a very small load at different times after stretch (load clamp). The shortening records derived after a preceding stretch exhibited a larger and steeper initial transient than that recorded in an isometric tetanus without stretch. The excess length change (L_S; nanometres per half-sarcomere) recorded during the initial transient increased with the amplitude of stretch and was linearly related to the force enhancement produced by the stretch (F_E; % of maximum tetanic tension) according to the following regression: $L_S = 0{\cdot}200\ F_E + 8{\cdot}65$ ($P < 0{\cdot}001$). The length changes recorded from the whole fibre agreed well with measurements from individual segments.

4. Slack-test measurements confirmed the existence of a large initial transient phase when the fibre was released to shorten after a preceding stretch. The excess length change determined from the slack tests agreed closely with the values derived from load-clamp recordings.

5. The results support the view that stretching a muscle fibre during tetanus leads to strain of elastic elements and, presumably, to variation of filament overlap due to non-uniform distribution of the length change within the fibre volume. Regions with greater filament overlap are likely to generate the long-lasting extra force referred to as 'residual force enhancement after stretch'. The elastic elements recruited during stretch can be presumed to play an essential part in this process by supporting regions in which the filament overlap has been reduced during the stretch ramp. Recoil of these elastic elements is responsible for the excess length change that is recorded during the initial transient after release as described under point 3.

Striated muscle that is subjected to stretch during tetanic activity increases its force above the level attained in an isometric tetanus at the corresponding sarcomere length (e.g. Fenn, 1924; Abbott & Aubert, 1952; Hill & Howarth, 1959; Sugi, 1972; Cavagna & Citterio, 1974; Edman, Elzinga & Noble, 1978; Julian & Morgan, 1979; Sugi & Tsuchiya, 1981; Noble, 1992 and further references therein). Previous studies on frog single muscle fibres (Edman *et al.* 1978) suggest that this force increment has the following two main components. (a) A velocity-dependent increase of force that is fully developed soon after the onset of stretch and remains throughout the stretch period. This component disappears gradually within 4–5 s after the movement has stopped at low (~2 °C) temperature. (b) A velocity-*independent* increase of force that persists to the end of a long tetanus, i.e. it remains after component (a) has disappeared (Edman *et al.* 1978; Edman, Elzinga & Noble, 1982). Component (b) is referred to as 'residual force enhancement after stretch' (Edman *et al.* 1978). It becomes manifest at sarcomere lengths greater than approximately 2·2 μm and it increases in magnitude with the amplitude of the stretch ramp.

Authors' names are in alphabetical order.

The velocity-dependent component of the force enhancement by stretch (component (a)) has been explored in considerable detail (Katz, 1939; Aubert, 1956; Edman *et al.* 1978; Flitney & Hirst, 1978; Sugi & Tsuchiya, 1981; Edman, Elzinga & Noble, 1981; Edman, 1988; Lombardi & Piazzesi, 1990; Stienen, Versteeg, Papp & Elzinga, 1991; Piazzesi, Francini, Linari & Lombardi, 1992; Curtin & Edman, 1994; Månsson, 1994). The experimental evidence suggests that this part of the force enhancement is due to increased strain of attached cross-bridges, most probably in combination with a slight increase in the number of attached bridges (Sugi & Tsuchiya, 1988; Lombardi & Piazzesi, 1990), as the sarcomeres are forcibly extended during tetanic activity. In contrast, the residual force enhancement after stretch (component (b)) has characteristics that would seem unrelated to the properties of the cross-bridges. For example, the residual force enhancement after stretch increases with decreasing filament overlap and, equally important, it is not affected by the velocity of the stretch (Edman *et al.* 1978, 1982). Furthermore, the total force never exceeds the isometric force recorded at optimal sarcomere length (Julian & Morgan, 1979; Edman *et al.* 1982) suggesting that the residual force enhancement after stretch is not a recruitment of additional contractile potential.

In the present study the force enhancement after stretch has been further explored in isolated muscle fibres of the frog using techniques that enabled measurements of force and length changes both from the fibre as a whole and from discrete segments along the intact fibre. The experiments have been designed with the specific aim of testing whether the force enhancement after stretch is associated with strain of passive elastic elements within the fibre. Some of the results have been presented before in a preliminary form (Tsuchiya & Edman, 1990).

METHODS

Preparation and mounting

Single fibres were isolated from the anterior tibialis muscle of cold-adapted *Rana temporaria*. The frogs were killed by decapitation followed by destruction of the spinal cord. Care was taken to remove adherent connective tissue from the fibres along their entire length. The fibres were mounted horizontally in a temperature-controlled Perspex chamber between a force transducer and an electromagnetic puller as previously described (Edman & Reggiani, 1984). With the approach used, the attachment of the fibre to the hooks of the force transducer and puller could be adjusted appropriately to make any vertical or lateral movements of the fibre insignificant during contraction. The experiments were carried out within a range of sarcomere lengths (2·05–2·65 μm) where the resting tension was negligible.

The bathing solution had the following composition (mM): NaCl, 115·5; KCl, 2·0; $CaCl_2$, 1·8; $Na_2HPO_4 + NaH_2PO_4$, 2·0; pH 7·0. The solution was pre-cooled and perfused through the muscle chamber (volume, *ca* 2·5 ml) at a speed of approximately 2·0 ml min^{-1}. The temperature of the bathing solution was kept constant to $\pm$0·1 °C during any given experiment but ranged between 0·5 and 3·6 °C in the whole series of experiments. Fibre length, sarcomere length (laser diffraction) and cross-sectional area were determined as described previously (Edman & Reggiani, 1984).

Stimulation

Rectangular current pulses (0·2 ms duration) were passed between two platinum plate electrodes that were placed symmetrically on either side of the fibre approximately 2 mm from it. The stimulus strength was 15–20% above the threshold. Tetani of 2–7 s were produced in different experiments and the stimulation frequency (16–20 Hz) was adjusted to provide complete, or nearly complete, fusion of the isometric force under the various conditions studied. The intervals between tetani were constant in any given experiment and varied between 5 min in experiments with relatively short (2 s) tetani and 10 min in experiments with tetani of long (7 s) duration.

Muscle chamber, force transducer and electromagnetic puller

A detailed description of the muscle chamber, the force transducer (AE801, Aksjeselskapet Mikroelectronikk, Horten, Norway) and the electromagnetic puller has been given earlier (Edman & Reggiani, 1984).

Segment length recording

Changes in length of marked segments of isolated muscle fibres were monitored using a modified version of the photoelectric recording device previously described (Edman & Reggiani, 1984). According to this method discrete segments, approximately 0·5 mm in length, were demarcated by means of opaque markers of black dog's hair or, in later experiments, rectangular pieces of letterpress (Edman & Lou, 1990). The markers were attached to the upper surface of the fibre in the bath, and their relative position was recorded by means of a photodiode array (Fairchild CCD133, Fairchild Corporation, Mountain View, CA, USA). An analog circuit provided a signal that was proportional to the change in length of one segment, i.e. to the change in distance between two adjacent markers. The accuracy of this measurement was better than 0·2% of the segment's length. The time resolution of the segment length measurement was 40 μs. The actual distance between the markers on the fibre was determined at ×40 magnification using a Zeiss stereomicroscope provided with an ocular micrometer.

Stretch ramps

The stretch ramp was applied in the beginning of the plateau of the isometric tetanus. Except for one series of experiments to be described separately (see Results), the amplitude of the stretch was 75 nm per half-sarcomere (h.s.), and the length change was performed over a time period varying between 300 and 600 ms in the different experiments (velocity of stretch, 125–250 nm s^{-1} h.s.$^{-1}$).

Analysis of data

The signals from the force transducer, the electromagnetic puller and the photodiode array were recorded and analysed using a Nicolet 4094B oscilloscope. In a few experiments the above signals were displayed and photographed on a Tektronix 5113 oscilloscope. In the latter case the film records were measured on a Nikon projector using the stage micrometer reading as previously described (Edman, 1979).

Maximum isometric force during a fused tetanus is referred to as P_0 and is used in several figures as a standard.

Student's *t* test was used for determination of statistical significance. All statistics are given as means $\pm$ S.E.M.

RESULTS

Figure 1 shows the response to a slow stretch of a single muscle fibre during tetanus at 1·90–2·05 μm (*A*) and at 2·50–2·65 μm (*B*) sarcomere length. The stretch ramp (amplitude, 75 nm h.s.$^{-1}$) was applied during the early plateau phase of the isometric tetanus and was performed over a time period of 300 ms, i.e. at a velocity of 250 nm s^{-1} h.s.$^{-1}$. In accordance with previous results (Edman *et al.* 1978, 1981), stretch during tetanic activity caused tension to rise rapidly and the tension remained high during the remainder of the stretch period. This increase in force is referred to as 'force enhancement during stretch'. After the end of stretch, tension declined to a steady level that was reached within 4–5 s. When stretch was performed near slack length (Fig. 1*A*) the final tension after stretch was not significantly different from that recorded during an ordinary isometric tetanus at the corresponding sarcomere length. However, when stretch was carried out well above slack length (Fig. 1*B*), there was a clear 'residual force enhancement after stretch' (Edman *et al.* 1978), i.e. the final tension after stretch exceeded the control isometric force at the same overall sarcomere length. The residual force enhancement after stretch has previously been shown to increase with the amplitude of stretch and to be independent, over a wide range, of the velocity at which the stretch is performed (Edman *et al.* 1978).

In the following experiments the fibre's ability to shorten against a very small load has been investigated at various levels of force enhancement after stretch. The main purpose of the experiments has been to test whether the force enhancement after stretch is associated with strain of elastic elements within the muscle fibre.

Velocity of shortening at zero load recorded after force enhancement by stretch

Load-clamp recordings

The velocity of shortening near zero load was recorded at different times after the end of stretch, i.e. at various degrees of decay of the force enhancement. Similar recordings were performed during control isometric tetani performed at the corresponding sarcomere length. The fibre was released to shorten under load-clamp control, the

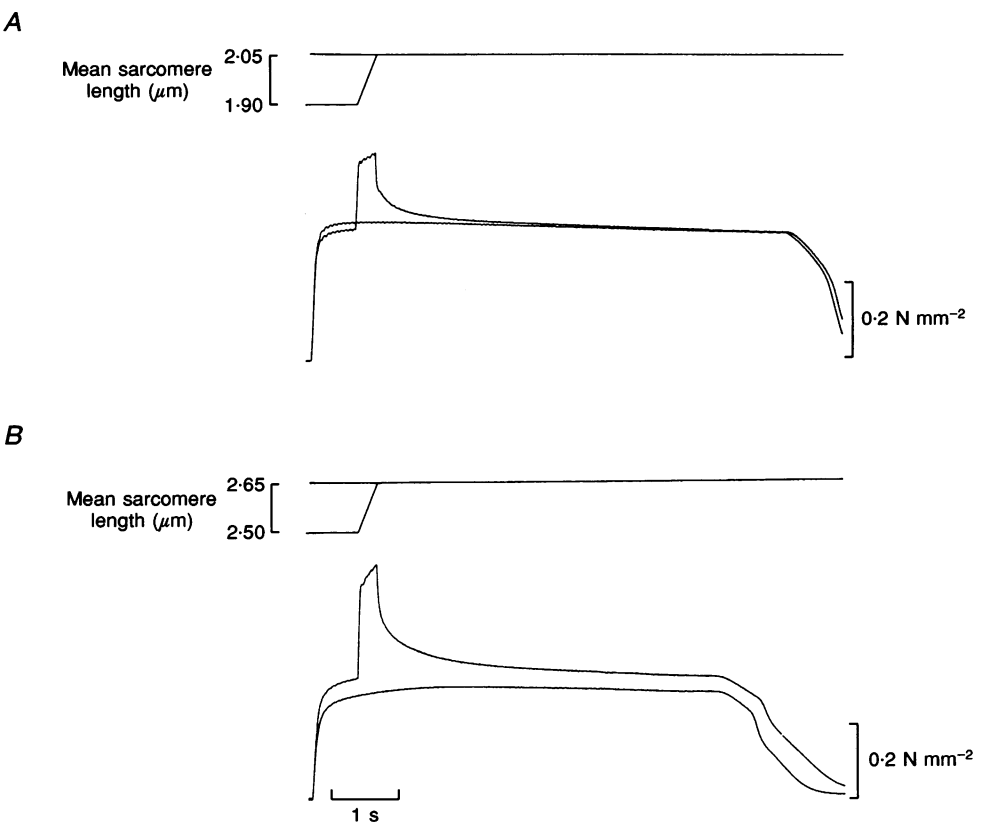

Figure 1. Force and displacement records from a single muscle fibre during tetani at two different sarcomere lengths

A, stretch during activity from 1·90 to 2·05 μm sarcomere length compared with ordinary isometric tetanus at 2·05 μm. *B*, comparison of stretch from 2·50 to 2·65 μm sarcomere length with isometric tetanus at 2·65 μm. Note that the same force level is finally reached in both records in *A*, whereas force after stretch in *B* remains above the isometric control level throughout the activity period. Temperature, 2·2 °C.

clamp level being set at 2–3% of the tetanic force at the sarcomere length considered. Shortening was recorded both from the fibre as a whole (puller movement) and from a marked segment of the fibre.

Figure 2 illustrates sample records from load-clamp recordings performed 5 ms after the end of stretch (*A*) and at the corresponding time during a control tetanus without stretch (*B*). The sarcomere length was adjusted to be the same at the onset of release in both *A* and *B*. Shortening records from the whole fibre and from a marked segment are shown together on a fast time base in *C* and *D*. The two records both exhibited a transient phase of rapid shortening after release as previously observed (e.g. Civan & Podolsky, 1966). This initial phase was succeeded by shortening at a lower, constant speed. The speed and amplitude of the initial transient phase were substantially larger during release after stretch than in the isometric control. This is clearly seen in Fig. 2*E* and *F* where control and test records from the whole fibre (*E*) and from a short segment of the same intact fibre (*F*) have been superimposed for comparison. It is noteworthy that the speed of shortening following the initial transient is nearly

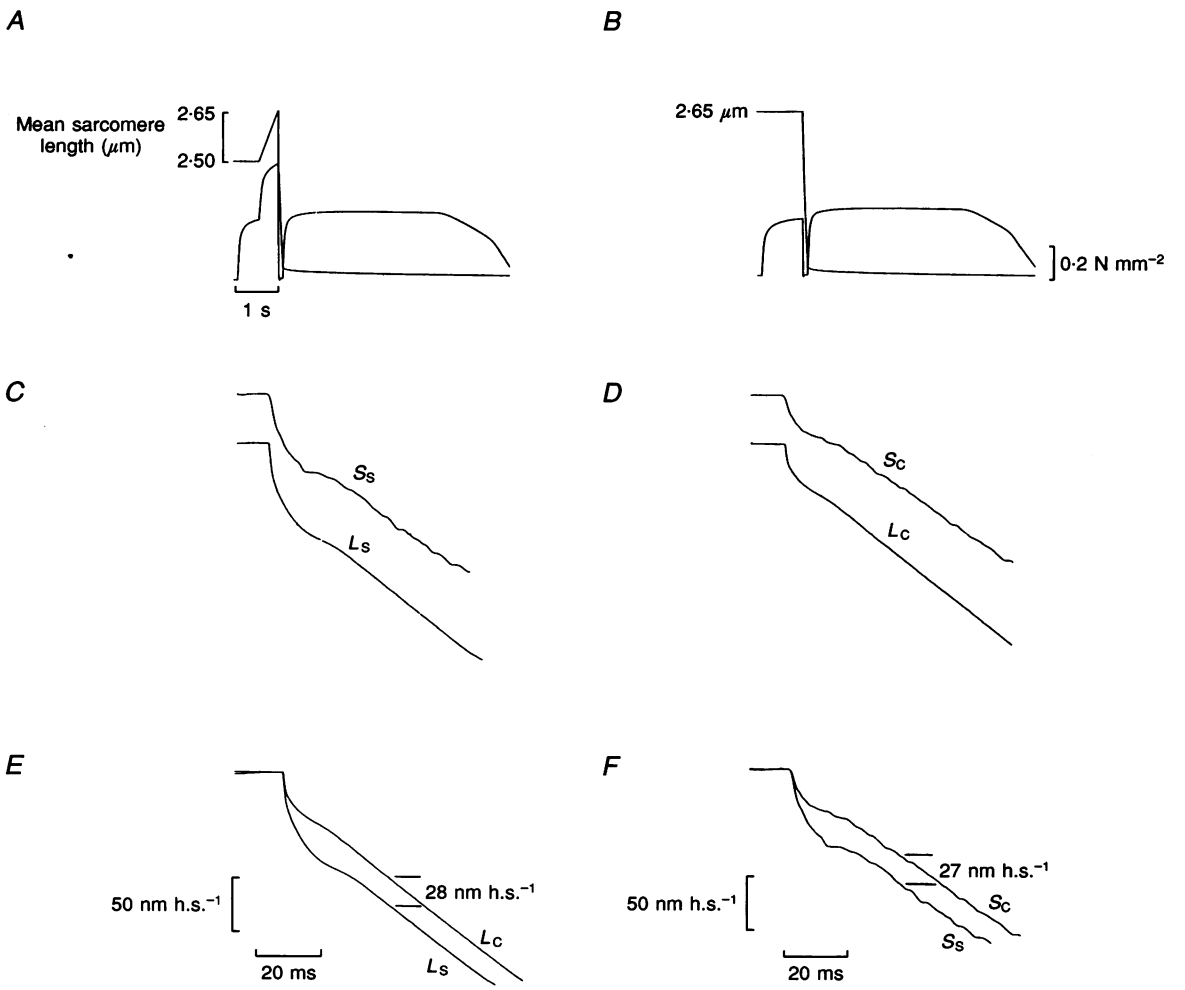

Figure 2. Example records of force and length changes of a single muscle fibre released to shorten against a small load during tetanus (load clamp)

A and *B*, force (lower) and displacement records (puller movement, upper) shown on a slow time base. In *A* the fibre is released immediately after a stretch ramp and in *B* (control) the release is performed at the corresponding time and sarcomere length without a preceding stretch. The overall sarcomere length is given at the displacement records. *C*, length changes of the whole fibre (L_S) and of a marked fibre segment (S_S) recorded on a fast time base during shortening after stretch. *D*, corresponding records of fibre length (L_C) and segment length (S_C) in control run. *E* and *F*, the shortening records derived after stretch in whole fibre and segment (L_S and S_S) superimposed on control records (L_C and S_C) for comparison. Note that the shortening records have an initial transient phase the amplitude of which is larger after a preceding stretch ramp. The vertical distance between the parallel length traces illustrated in *E* and *F* provides a measure of the excess length change that occurs during the initial transient after a preceding stretch. The scale bars in *E* and *F* also apply to *C* and *D*. Temperature, 1·0 °C.

the same in test and control. The ratio of the shortening velocities measured after the initial transient in test and control runs was 0·998 ± 0·005 based on twenty-nine paired observations in six fibres. The corresponding value derived from segment measurements was 1·003 ± 0·007 ($n = 12$, 3 fibres). Because of this similarity in speed of shortening in the presence and absence of stretch the vertical distance between the test and control records (E and F) provides a convenient measure of the difference in amplitude of the initial transient phase. This difference was measured 30–50 ms after the release and is referred to below as the *excess length change induced by stretch.*

Shortening records at two different sarcomere lengths (2·20 and 2·65 μm) derived immediately after stretch and, at the corresponding time, during an isometric tetanus are shown superimposed in Fig. 3. The time course of shortening after release during isometric tetanus without preceding stretch (length traces *a* and *b*) was quite similar at the two sarcomere lengths, the amplitude of the initial transient being virtually identical in the two cases. By contrast, the speed and amplitude of the initial transient during shortening after stretch were considerably larger at the greater sarcomere length (cf. length traces *c* and *d*).

The excess length change induced by stretch, as defined above, was investigated at different times after the end of stretch and was related to the amplitude of the force enhancement that existed when the fibre was released (see inset of Fig. 4*A*). Results from such measurements derived from one fibre are presented in the main diagram of Fig. 4*A*. The data are based on recordings both from the whole fibre and from a short segment of the same fibre at 2·20 and 2·65 μm sarcomere length at three different times after the end of stretch. The time of release after the end of stretch (in seconds) is indicated at the respective data point. A similar plotting including data from six different fibres is shown in Fig. 4*B*.

The results obtained from whole fibres and individual segments can be seen to agree well. They both indicate that the excess length change induced by stretch was linearly related, over a wide range, to the force enhancement existing at the moment when the fibre was released. A linear regression of the excess length change induced by stretch (L_S) upon the force enhancement by stretch (F_E) derived from all points in Fig. 4*B* provides the following relationship:

$$L_S = 0{\cdot}200\ F_E + 8{\cdot}65.$$

The slope of this line is different from zero with a P value $< 0{\cdot}001$. The relation between L_S and F_E for very low values of F_E cannot be assessed from the present data. The relation is likely to be curved in this region and to extend to the origin as suggested by the dashed line in

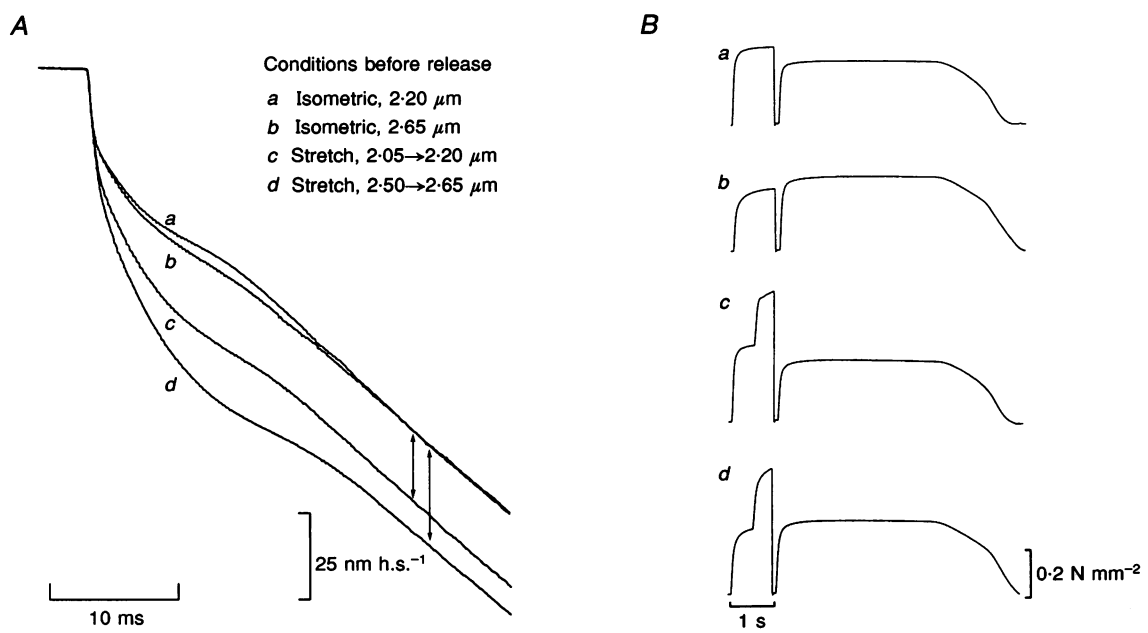

Figure 3. Superimposed length records from a single muscle fibre released to shorten against a small load during tetanus

A, superimposed length traces (*a–d*). *B*, corresponding force records. Traces *a* and *b*, release during control isometric tetanus at 2·20 and 2·65 μm sarcomere length, respectively. Traces *c* and *d*, release immediately after a stretch ramp from 2·05 to 2·20 (*c*) and from 2·50 to 2·65 μm sarcomere lengths (*d*). Note that the slope of the shortening records, after the initial transient has passed, is quite similar in *a–d*. The amplitude of the initial transient, indicated by the vertical shift of the superimposed traces (arrows), is increased by a preceding stretch, the more so the greater the sarcomere length at which the stretch is performed (cf. traces *c* and *d*). Fibre length at 2·20 μm sarcomere length, 6·9 mm. Temperature, 1·0 °C.

Fig. 4*B*. A non-linear length–tension relationship in the low-force range does seem to be a characteristic feature of the passive elastic structures in skeletal muscle (e.g. Jewell & Wilkie, 1958; Cleworth & Edman, 1972; Wang, McCarter, Wright, Beverly & Ramirez-Mitchell, 1993).

The excess length change induced by stretch increased in size with the amplitude of the preceding stretch. This is demonstrated in Fig. 5, which shows measurements performed soon after the end of a stretch ramp during the tetanus plateau using the procedure described above. The tetanus was initiated at sarcomere lengths between 2·05 and 2·20 μm and the fibre was stretched during the plateau phase to 2·25 μm in the various runs (velocity of stretch, 188 nm s^{-1} h.s.$^{-1}$). An isometric tetanus without stretch but including a load-clamp recording was performed at 2·25 μm sarcomere length. The results show that, within the range investigated, the excess length change induced by stretch was steadily increased as the amplitude of stretch was made larger. The linear regression shown in Fig. 5 gives the following relation between the excess length

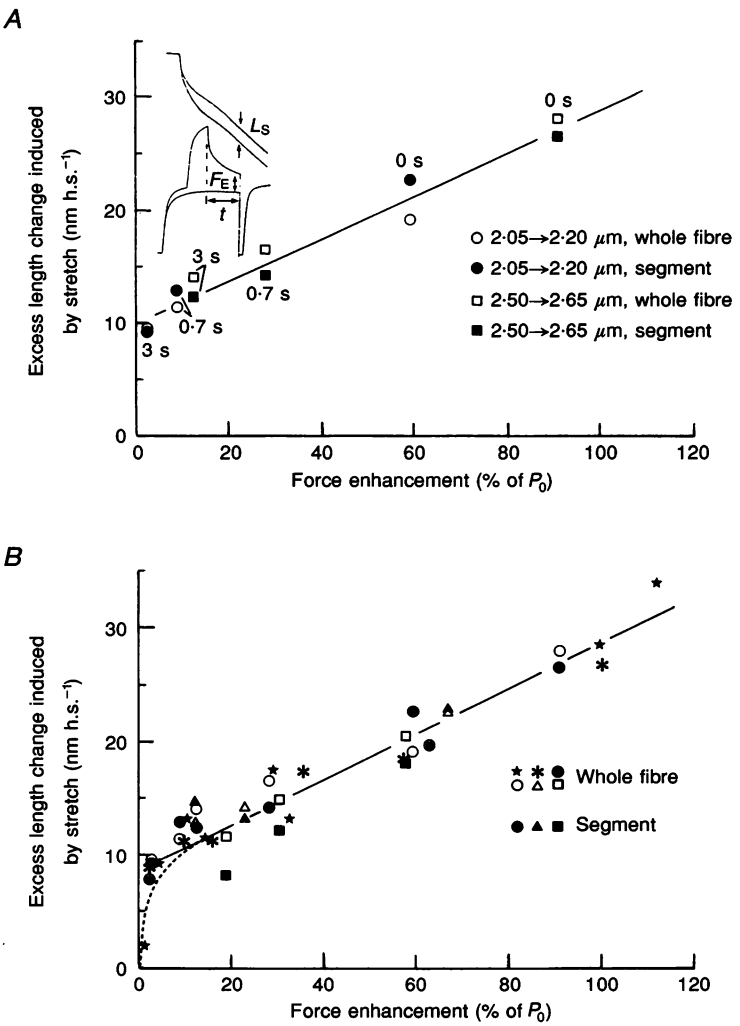

Figure 4. Relation between force enhancement after stretch (F_E) and the excess length change during shortening after a preceding stretch (L_S)

A and *B* are load-clamp recordings. The inset shows superimposed traces of fibre length (upper traces) and tension (lower traces) during load-clamp recording after a preceding stretch and in a control tetanus without stretch. *A*, data derived from one preparation with measurements from the whole fibre and from a discrete segment as indicated by the symbol key. The latter also gives the range of sarcomere lengths over which the fibre was stretched before release. The time (t, inset) from the end of stretch to the onset of release is given in seconds at the data points. Line, least-squares regression of L_S upon F_E. *B*, data from 6 fibres including the one shown in *A*. Measurements from whole fibre and segment as indicated by symbol key. Continuous line, linear regression based on all data points: $L_S = 0{\cdot}200\ F_E + 8{\cdot}65$. Dashed line, fitting by eye of data in the low-force range to make the length–tension relation start from the origin.

change induced by stretch (L_S) and the amplitude of the preceding stretch (S_A):

$$L_S = 0{\cdot}409\ S_A + 9{\cdot}49\ (P < 0{\cdot}001,\ n = 11).$$

The amount of stretch required to reach the transition between the initial steep phase of tension rise and the slow component of tension rise during stretching has previously been found to be approximately 16 nm h.s.$^{-1}$ in measurements on whole fibres (Edman *et al.* 1981) and 12–14 nm h.s.$^{-1}$ at an equivalent stretch velocity in measurements on fibre segments (Lombardi & Piazzesi, 1990). It can be deduced from the regression shown in Fig. 5 that a stretch amplitude of this size, ~14 nm h.s.$^{-1}$, is associated with an 'excess length change induced by stretch' of similar magnitude, ~15 nm h.s.$^{-1}$.

Slack tests

Slack test recordings (Edman, 1979) were used as an alternative method of measuring the excess length change induced by stretch. The fibre was slackened by a given amount during isometric activity to shorten at maximum speed and redevelop tension at the new length. Three or more amplitudes of release were used in these experiments and the time (Δt) taken from the onset of release to the onset of force redevelopment was measured in each case. The amplitude of release (ΔL) ranged between 3 and 8% of the fibre length. A series of slack tests was performed at a given time after a stretch and, in a corresponding manner, during a control tetanus. The releases were carried out, with or without preceding stretch, at 2·65 μm sarcomere length. Typical plots of slack-test data derived after stretch and during an isometric control tetanus are shown in Fig. 6*A* and *B*. For each set of data points, a least-squares regression of ΔL upon Δt was calculated. The slope of this regression is a measure of the speed of shortening at zero load, V_0 (Edman, 1979). This measurement can be presumed to be little affected by the initial length transient that occurs after release. This is inferred from the fact that the initial transient would be completed before the end of the slack period for the smallest release step used. In accordance with this view, the slope of the $\Delta L/\Delta t$ relation was not found to differ in any systematic way when slack-test measurements were performed after stretch and in the absence of preceding stretch, respectively. The ratio between the V_0 values recorded after stretch and in the absence of stretch was 1·068 ± 0·029 (paired observations, 5 fibres, not significantly different from unity).

The upward shift of the $\Delta L/\Delta t$ relation observed after stretch (Fig. 6*A* and *B*) may be attributed to the fact that there was a larger initial length transient when the fibre was released after stretch. The magnitude of this shift was thus used as an alternative measure of the excess length change induced by stretch that was previously defined under 'Load-clamp recordings'. This measurement was carried out as illustrated in Fig. 6*A* and *B*, i.e. at an intermediate slack time (near 20 ms) in the control series.

The results obtained from the slack tests are summarized in Fig. 7 and can be seen to be in excellent agreement with the data derived in the load-clamp experiments (cf. regression lines in Figs 4*B* and 7). Both sets of data show that force enhancement by stretch to a level 100% above the isometric control was associated with an excess length change after release of 28 nm h.s.$^{-1}$.

Relation between the slow component of force enhancement during stretch and the residual force enhancement after stretch

As previously demonstrated (Edman *et al.* 1978; see also Fig. 1) the force enhancement *during* stretch contains an initial rapid phase that is succeeded by a slow phase of tension rise during the remainder of the stretch period. The transition between the two phases of force enhancement during stretch is often quite distinct, with the formation of a break-point in the force myogram. The second, slow phase will be referred to as the '*slow component of force*

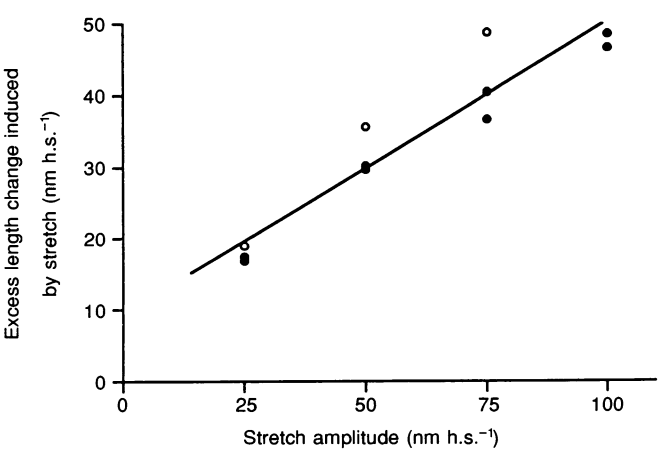

Figure 5. Relation between amplitude of stretch (S_A) and excess length change induced by stretch (L_S)
The data refer to two single muscle fibres (identified by different symbols). Whole-fibre recordings: sarcomere length at which the stretch ramp ended, 2·25 μm. Line, linear regression: $L_S = 0{\cdot}409\ S_A + 9{\cdot}49$. For further information, see text. Temperature, 0·6–1·2 °C.

enhancement during stretch'. The amplitude of this component is small when stretch is performed near slack fibre length but increases at greater lengths (Edman *et al.* 1978). Earlier experiments have demonstrated (Edman, Elzinga & Noble, 1984) that the slow component of force enhancement during stretch and the residual force enhancement after stretch both increase with the amplitude of stretch, suggesting that the two phenomena are somehow related. The following experiments were designed to further elucidate the interrelationship between the above two components of force enhancement by stretch.

The experimental protocol used for stretching the fibre during tetanic stimulation was essentially the same as that described in the foregoing sections. In the present set of experiments tetani of 7 s duration were initiated at four different sarcomere lengths, 2·50, 2·55, 2·60 and 2·65 μm in repeated runs, and the stretch amplitude was adjusted to give the same final sarcomere length, 2·7 μm, at the end of stretch in each case. A control tetanus without stretch was performed at 2·7 μm sarcomere length after each series of four stretch runs.

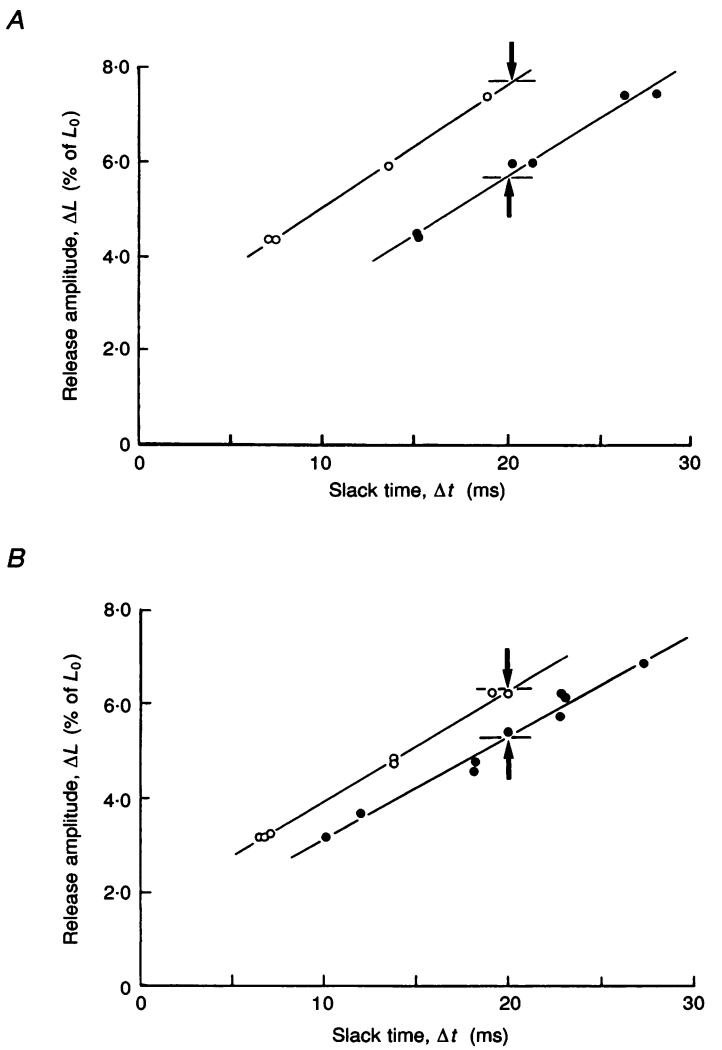

Figure 6. Slack test diagrams illustrating the relation between amplitude of shortening (ΔL) and time from onset of release to beginning of force redevelopment (Δt)

Contractions were initiated at 2·50 μm sarcomere length and the fibre was stretched to 2·65 μm during the tetanus plateau. *A*, fibre was released immediately after the stretch ramp. *B*, fibre released 2 s after the end of stretch. ○, releases after stretch. ●, releases not preceded by stretch ramp in a tetanus at 2·65 μm sarcomere length. The vertical distance between the regression lines is measured at an intermediate slack time in the control (indicated by arrows). The measured vertical distance between the lines is 27 nm h.s.$^{-1}$ in *A* and 12·5 nm h.s.$^{-1}$ in *B*. Note that: (i) the slack test relation is shifted towards greater values of ΔL when releases are preceded by stretch and (ii) the shift is less pronounced when the slack tests are performed at a later time after stretch. Temperature, 3·6 °C.

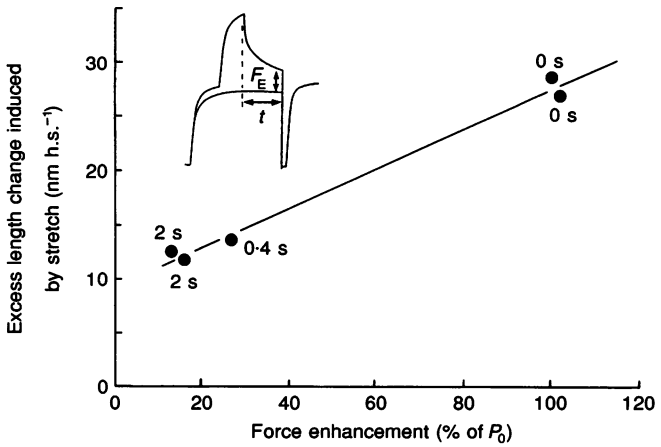

Figure 7. Relation between force enhancement after stretch (F_E) and excess length change during shortening after a preceding stretch (L_S)

Slack test recordings at 2·65 μm sarcomere length. Inset, superimposed force records of slack tests after a preceding stretch with force enhancement and during a control tetanus without stretch. The time (t, inset) from the end of stretch to the onset of release is given in seconds at the symbols. Line, linear regression: $L_S = 0{\cdot}184\, F_E + 9{\cdot}19$.

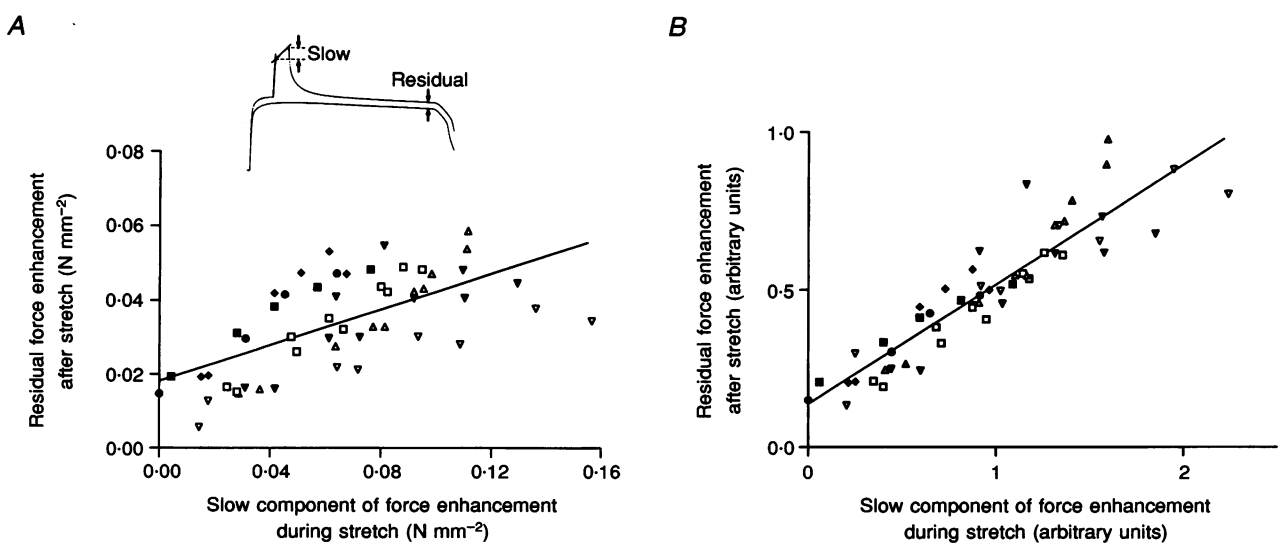

Figure 8. Relation between slow component of force enhancement during stretch and residual force enhancement after stretch studied in seven isolated muscle fibres

A, inset: tetanus with force enhancement by stretch superimposed on control tetanus to illustrate the approach used for measuring the slow component of force enhancement during stretch ('slow') and the residual force enhancement after stretch ('residual'). Values from a given fibre denoted by the same symbol. Line, linear regression based on all data points ($P < 0{\cdot}0001$, $n = 53$). Force expressed in newtons per millimetre squared. *B*, data in *A* replotted after normalization to a point on the regression line in *A* that corresponds to 0·07 N mm^{-2} on the abscissa. For this transformation the residual force enhancement after stretch was first determined (by interpolation) at 0·07 N mm^{-2} in the individual experiment. The ratio between this value and the corresponding value of the residual force enhancement given by the regression line in Fig. 8*A* was then used to transform the values in the respective set of data. One unit on abscissa and ordinate corresponds to 0·07 N mm^{-2}. Line, linear regression ($P < 0{\cdot}0001$). Note that the residual force enhancement after stretch is correlated, with high statistical significance, to the slow component of force enhancement during stretch.

Figure 8A, which summarizes the results of seven experiments, shows that the slow component of force enhancement during stretch and the residual force enhancement after stretch were closely related to one another albeit with a relatively large interfibre variability. In Fig. 8B this interfibre variability has been accounted for by normalizing the data in the various experiments to a point on the linear regression in Fig. 8A that corresponds to 0·07 N mm^{-2} on the abscissa, i.e. to a point near the median of the data set displayed in Fig. 8A. The replotted data in Fig. 8B show a good agreement between the individual experiments with respect to the relative size of the two components of force enhancement by stretch. According to the linear regression shown in Fig. 8B, the residual force enhancement after stretch was approximately half the size of the slow component of force enhancement during stretch, except for measurements derived after very small stretches (lower left part of the diagram).

DISCUSSION

Strain of parallel elastic elements during stretch of active muscle fibre

The present study concerns the long-lasting enhancement of force that occurs when striated muscle is slowly stretched above optimum length during tetanic activity. This increase of force, referred to as 'residual force enhancement after stretch' (Edman *et al.* 1978) is maintained, without appreciable decay, during several seconds of tetanic stimulation. The phenomenon, first observed in frog whole skeletal muscle (Fenn, 1924; Abbott & Aubert, 1952; Hill & Howarth, 1959; Cavagna & Citterio, 1974; Amemiya, Iwamoto, Kobayashi, Sugi, Tanaka & Wakabayashi, 1988), has been characterized in considerable detail in studies of single muscle fibres (Hill, 1977; Edman *et al.* 1978, 1982; Julian & Morgan, 1979; Sugi & Tsuchiya, 1988) including measurements on short, length-clamped segments of the intact fibre (Edman *et al.* 1982). As pointed out previously (Edman *et al.* 1978, 1982), the residual force enhancement after stretch has features compatible with the idea of a force being recruited from a parallel elastic unit. In line with this view the residual force enhancement after stretch is not influenced by the velocity of stretch but is critically dependent on the stretch amplitude. The present results provide evidence that force enhancement by stretch is indeed associated with recruitment of damped elastic elements. The data suggest that strain of the elastic components plays an essential part in the development of force enhancement after stretch. The following observations are pertinent to the conclusion that passive elastic elements do arise during stretch.

When a fibre is released to shorten against a small load after stretch there is an initial transient phase during which the fibre shortens at a high speed. The amplitude of this transient phase increases with the magnitude of force enhancement after stretch (Fig. 4). This initial shortening phase is likely to involve recoil of passive structures along the muscle fibre, i.e structures previously extended during the stretch ramp. These passive components, by exerting a longitudinal compressive force upon the sarcomeres (see further below), will increase the speed of shortening when the fibre is released against a small load. After the initial transient has passed, and the elastic component can be presumed to have recoiled fully, the shortening velocity settles at a value that is indistinguishable from that recorded in a control run without stretch. Our data suggest that a substantial amount of elastic strain (~15 nm h.s.$^{-1}$) is already present at the end of the steep phase of force enhancement during stretch, a point reached by a stretch ramp approximately 12–16 nm h.s.$^{-1}$ in amplitude (Edman *et al.* 1981; Lombardi & Piazzesi, 1990). The strain of the elastic elements increases steadily with increasing amplitude of stretch (Fig. 5). After the end of the stretch ramp the strain is partially reversed in parallel with the decrease in force.

The relatively long time required for completion of the initial transient after release is evidence against the idea that the transient is due to the recoil of undamped elastic elements. The transient covers a time period of 10–15 ms and has the character of a damped movement (Fig. 3). An initial, relatively fast, length step indicative of recoil of an undamped series elastic element does appear in length records derived from the whole fibre (Fig. 3). This rapid length change, which may be attributed to recoil of tendinous structures at the fibre ends, was not, however, clearly distinguishable in the segment-length records. Its presence in the whole-fibre recordings did not affect the measurement of the 'excess length change induced by stretch' as indicated by the good agreement between the results derived from whole fibres and segments. This is explainable by the fact that the tendon compliance is small in the high-force range (Mason, 1978).

Origin of elastic elements affected during stretch

The experiments were performed at sarcomere lengths where resting tension was zero. The structure from which the elastic elements are recruited during stretch evidently has to be reorganized or realigned during activation or during the stretch itself.

Electron microscopical evidence would seem to make clear that neighbouring myofibrils within a muscle fibre are intimately connected to one another by passive structures that make up the cytoskeletal matrix. Connections exist between adjacent myofibrils at each Z-line and M-line (Garzia-Nunzi & Franzini-Armstrong, 1980; Brown & Hill, 1982; Wang & Ramirez-Mitchell, 1983) explaining the remarkable order of the striation pattern at rest throughout the fibre volume. The lateral connections at the Z- and

M-lines are linked together with longitudinal components made up of connectin (also named titin) and nebulin filaments (Maruyama *et al.* 1977; Wang *et al.* 1993). Each myofibrillar sarcomere thus contains a meshwork of passive structures which serves to keep the myofibrils in register along the fibre at rest and during activity. These passive structures are likely to become further strained during the stretch ramp. Our results do support the view that elastic components are already being strained from the outset of stretch (see Fig. 5 and corresponding text).

The development of elastic components during isometric activity is explainable by the fact that adjacent myofibrils regularly undergo a slight longitudinal shift relative to each other when the fibre contracts while the ends are fixed. Such a shift of the myofibrils is readily seen as a staggering of adjacent myofibrillar sarcomeres when the fibre is viewed in a light microscope during tetanus at an appropriate (× 200–400) magnification. The shift is small, generally less than the length of a sarcomere. An increased degree of staggering of adjacent myofibrils during tetanic activity, often in combination with skewing of Z- and M-lines, is likewise demonstrable in electron micrographs pictures of fibres that have been quickly frozen during the tetanus plateau (K. A. P. Edman & F. Lou, unpublished results). These changes may be thought to arise because of differences in contractile strength among the fibrils along the length of the fibre. When the fibre is stretched during tetanus, these pre-extended elastic structures will be further strained.

Brown & Hill (1991), studying the variation in filament overlap in fibres rapidly fixed in a mercuric chloride ethanol–chloroform medium, reported that in fibres stretched during a tetanus there were areas with grossly uneven half-sarcomeres. That is, even though the overall sarcomere length remained fairly uniform within the fibre volume, there was a considerable degree of staggering of the thick filaments, resulting in marked irregularity of filament overlap within the two halves of a myofibrillar sarcomere. These observations are unlikely to be fully applicable to the present results as the stretch ramps used by Brown & Hill (1991) were larger in amplitude and, most importantly, had velocities that were more than fifty times higher than those employed here. However, some irregularity of filament overlap within individual sarcomeres is normally seen in various places along the fibre, both during an ordinary isometric tetanus and after a stretch ramp of moderate speed and amplitude performed on the tetanus plateau (K. A. P. Edman & F. Lou, unpublished observations in fibres fixed by rapid freezing). The longitudinal shifts of the thick filaments within the sarcomere can be presumed to strain the connections between the M-lines of adjacent myofibrils, thus adding to the effect produced by the longitudinal displacement of the myofibrils discussed before.

A network of strained elastic components is thus likely to develop during stretch due to non-uniform distribution of the length change within the fibre volume. It is worth pointing out that the individual components of this network will act in series with neighbouring strong myofibrillar segments. However, each passive component will, at the same time, act in parallel with the weaker segment of the same myofibril and with other myofibrils across the fibre. Consequently, when the fibre is released to shorten against a small load during activity, the recoil of the passive structures will be hampered due to the limited speed of shortening of the myofibrils. This accords well with the damped character of the initial transient after release described in Fig. 3.

There is reason to believe that part of the excess length change induced by stretch is due to viscoelastic recoil of stretched cross-bridges. This fraction of the measured recoil is likely to amount to 3–4 nm h.s.$^{-1}$ according to results presented by Piazzesi *et al.* (1992). These authors determined the 'T_2 curve' (Ford, Huxley & Simmons, 1977) after stretch ramps (~30 nm h.s.$^{-1}$) that were more than sufficient to complete the steep phase of the force enhancement during stretch in discrete segments of frog muscle fibres. The intersection of the T_2 curve with the length axis may be considered to be a measure of the viscoelastic recoil of the cross-bridges when the force is reduced to zero level by a quick length step. The 3·1 nm h.s.$^{-1}$ shift of the T_2 curve observed by Piazzesi *et al.* (1992) after a stretch ramp thus represents the greater amount of extension of the bridge elasticity (including the change in attitude of the cross-bridge head) that is produced by the stretch. It is of interest to note in this connection that the recoil of the cross-bridge viscoelasticity is completed in 3 ms or less as suggested by the T_2 analysis. In contrast, the excess length change induced by stretch described in the present paper covers a time period of more than 15 ms, reflecting the additional, much larger recoil of damped elastic elements outside the cross-bridge domain when the fibre is released to shorten against a small load.

Does strain of elastic elements contribute the extra force recorded during and after stretch?

The excess length change induced by stretch was found to increase with the amplitude of stretch and to decrease successively after the end of the stretch ramp. These features of the excess length change do not accord with the stretch-induced changes in force. As pointed out before, the main portion of the force enhancement during stretching, i.e. the enhancement measured at the breakpoint of the force myogram, varies with the velocity of stretch and is independent of the stretch amplitude once the stretch ramp exceeds 12–16 nm h.s.$^{-1}$ (for references, see Introduction). Furthermore, the excess length change induced by stretch was not fundamentally different when measurements were

made near slack fibre length and at a longer sarcomere length, i.e. under conditions where the residual force enhancement after stretch was greatly different. Strain of passive elastic components during stretch apparently does not itself explain the extra force recorded during and after stretch. This is in line with the view (see above) that the extended elastic elements are dispersed within the fibre volume and that the force held by these elements only serves to support the weak part of the myofibril at which they are located. Thus from a functional point of view, the elastic components dealt with in the present study do not form a true parallel elastic element like that depicted in Fig. 9*A*. In the latter case, any strain of the parallel elastic component would contribute a force that is added to that produced by the active unit, and our results clearly show that this is not the case, as pointed out above. Fig. 9*B* illustrates a mechanical analog of the presumed functional arrangement of the elastic components that are responsible for the excess length change induced by stretch. According to this model, stronger and weaker myofibrillar segments act in series. The contractile unit of the weaker segment (CE_w) is supported by an elastic element (SE) that consequently acts in series with the contractile unit of the stronger segment (CE_s). The elastic element SE, being parallel to CE_w (and to contractile units of adjacent myofibrils), will have damped elastic properties.

Mechanism underlying the long-lasting component of force enhancement after stretch

Since strain of elastic elements alone cannot explain the extra force recorded during and after stretch, the long-lasting ('residual') force enhancement after stretch must be generated within the myofilament system itself. As pointed out in the preceding discussion, the recruitment of passive elasticity during stretch is most probably based on non-uniform length changes within the fibre volume during the stretch ramp. The non-uniform length changes lead to differences in filament overlap along the length of the myofibrils with variable amounts of staggering of the thick filaments within myofibrillar sarcomeres (see earlier). The non-uniformity of filament overlap is likely to be the main cause of the long-lasting force enhancement induced by stretch, as previously suggested by Morgan (1990, 1994).

The redistribution of filament overlap will increase the fibre's capacity to produce force when recordings are made above optimum fibre length (see further the Appendix in Edman & Reggiani, 1984). Regions that have acquired a greater amount of filament overlap will thus tend to increase the force above the control level. The elastic elements formed during the stretch ramp are here likely to play an essential role by supporting the weaker regions of the myofibrils. Strain of elastic elements may thus provide a mechanism by which the weakened parts of the myofibrils are appropriately supplemented with a parallel elastic force to enable them to match the stronger parts of the fibre during and after stretch. This accords with the finding that there is a continuous slow climb of tension during the stretch ramp when the fibre is extended above optimum length. The slow component of tension rise during stretch will remain, with some decrement, throughout the contraction period and will form the residual force

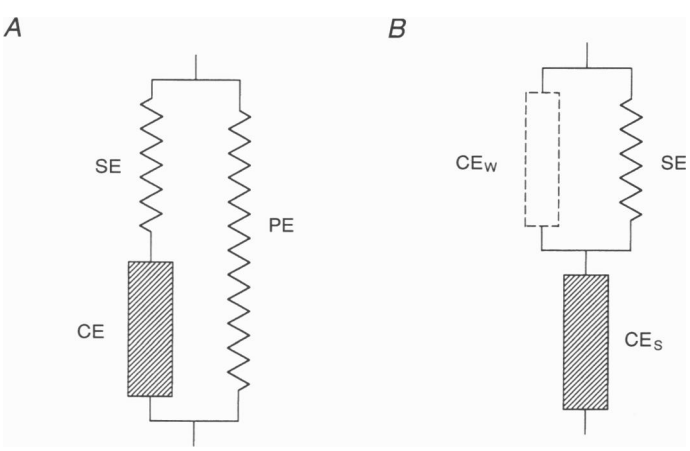

Figure 9. Schematic illustration of the functional arrangement of elastic and contractile elements in the muscle fibre

A, model in which the contractile element (CE) is assumed to act in series with an undamped elastic element (SE) with a second elastic element (PE) coupled in parallel with both CE and SE. *B*, schematic illustration of the proposed mechanical arrangement of the elastic elements responsible for the excess length change induced by stretch. A stronger contractile element (CE_s) here acts in series with a weaker contractile element (CE_w). The latter is supported by an elastic element (SE) that acts in series with CE_s.

enhancement after stretch, i.e. the increase in force that remains after the *velocity*-dependent component of force enhancement during stretch has disappeared fully. Our data suggest that the slow component of tension rise during stretch progressively declines after the end of the stretch ramp, reaching approximately half its original value at the end of the tetanus (see Fig. 8). This partial decline of the force enhancement may be due to a redistribution of the elastic strain along the myofibrils after the end of the stretch in line with the finding that the excess length change induced by stretch is steadily reduced after stretch (see Results).

The above mechanism offers a ready explanation for the long-standing observation that stretch does not lead to a greater force output than that recorded during an ordinary isometric tetanus at optimal sarcomere length (e.g. Edman *et al.* 1978, 1982; Julian & Morgan, 1979). The enhanced force recorded after stretch above slack length may thus approach, but never exceed, the isometric force at the peak of the length–tension relation. On the same ground, and in accordance with previous experimental findings (Edman *et al.* 1978), no residual force enhancement after stretch can be expected near slack fibre length.

The proposed mechanism of force enhancement finally explains the characteristic shift of the force–velocity relation that occurs after a stretch ramp. As demonstrated in both whole muscle (Cavagna & Citterio, 1974) and single muscle fibres (Edman *et al.* 1978; Sugi & Tsuchiya, 1981), force enhancement after stretch is associated with a shift of the force–velocity relation towards higher force values with no change in the measured value of the maximum speed of shortening, V_{max}. The shift of the force–velocity curve is related to the residual force enhancement after stretch, which means that the rightward shift of the high-force end of the curve is substantial after a stretch ramp above slack length but negligible after a stretch performed near optimal fibre length (Edman *et al.* 1978). The observed change of the force–velocity curve after stretch accords well with the mechanism of force enhancement proposed above, since regions with improved filament overlap after stretch will increase the isometric force and the fibre's ability to lift a load without affecting the measured value of V_{max} (Edman, 1979).

In a recent paper Cavagna, Heglund, Harry & Mantovani (1994) report that muscle fibres, stretched during tetanic activity, undergo a transient damped shortening when the tension is suddenly lowered after the stretch ramp and held (under load-clamp conditions) at the force level existing just before the stretch. The authors make a special point of the fact that: (1) the transient shortening occurs at sarcomere lengths near optimal length for tetanic force; (2) the transient shortening appears both at whole-fibre level and in discrete segments (~1 mm in length) of the fibre; and (3) the velocity of the transient shortening has a high Q_{10} value (~2·5). Based on their results Cavagna *et al.* (1994) put forward the idea that the increased ability of the muscle to shorten against the high load after stretch is due to release of mechanical energy stored 'within the damped element of the cross-bridges'.

The experimental evidence suggests that the 'transient shortening' observed by Cavagna *et al.* (1994) and the 'excess length change induced by stretch' described in the present paper both reflect the same underlying process, i.e. recoil of elastic elements that have been strained during the preceding stretch ramp. As demonstrated by the present results, strain of elastic elements during stretch is not confined to the descending limb of the length–tension relation but occurs at optimal fibre length as well. Furthermore, strain of elastic elements is demonstrable in segments along the entire fibre. The high Q_{10} value of the transient shortening reported by Cavagna *et al.* (1994) is also fully consistent with the idea that elastic recoil is the main cause of the transient shortening after stretch observed in their experiments, since the speed of elastic recoil is determined by the speed of shortening of contractile elements acting in parallel (see Discussion under 'Origin of elastic elements affected during stretch').

For several reasons the mechanism proposed by Cavagna *et al.* (1994) seems inadequate for explaining the transient shortening described by the latter authors. As pointed out in the preceding discussion, the elastic energy stored in the cross-bridges after a stretch ramp is fully discharged by a release step that is merely 3–4 nm h.s.$^{-1}$ larger than the T_2 value recorded in a control tetanus without stretch (Piazzesi *et al.* 1992). Furthermore, as may be inferred from the T_2 analyses (Piazzesi *et al.* 1992), the recoil of the cross-bridge viscoelasticity is completed within less than 3 ms, whereas 100 ms or more is required to complete the transient shortening recorded by Cavagna *et al.* (1994) at the high load considered. Another strong argument against the idea that stored elastic energy in the cross-bridges accounts for the force enhancement and the increased shortening potential after stretch is provided by the finding that both these changes become more pronounced above slack length, i.e. under conditions where the amount of filament overlap is reduced. In order to explain this aspect of the stretch effect on the basis of increased potential energy of the cross-bridges, one would have to assume that cross-bridges change their mechanical properties depending on the site along the thin filament where they attach. The experimental evidence obtained so far gives no reason to believe that this would be the case (see further Huxley, 1980).

ABBOTT, B. C. & AUBERT, X. M. (1952). The force exerted by active striated muscle during and after change of length. *Journal of Physiology* **117**, 77–86.

AMEMIYA, Y., IWAMOTO, H., KOBAYASHI, T., SUGI, H., TANAKA, H. & WAKABAYASHI, K. (1988). Time resolved X-ray diffraction studies on the effect of slow length changes on tetanized frog skeletal muscle. *Journal of Physiology* **407**, 231–241.

AUBERT, X. (1956). Le Couplage Energetique de la Contraction Musculaire. Thèse d'Agrégation de l'Enseignement Supérieur. Éditions Arscia, Brussels, Belgium.

BROWN, L. M. & HILL, L. (1982). Mercuric chloride in alcohol and chloroform used as a rapidly acting fixative for contracting muscle fibres. *Journal of Microscopy* **125**, 319–336.

BROWN, L. M. & HILL, L. (1991). Some observations on variations in filament overlap in tetanized muscle fibres and fibres stretched during a tetanus, detected in the electron microscope after rapid fixation. *Journal of Muscle Research and Cell Motility* **12**, 171–182.

CAVAGNA, G. A. & CITTERIO, G. (1974). Effect of stretching on the elastic characteristics and the contractile component of frog striated muscle. *Journal of Physiology* **239**, 1–14.

CAVAGNA, G. A., HEGLUND, N. C., HARRY, J. D. & MANTOVANI, M. (1994). Storage and release of mechanical energy by contracting frog muscle fibres. *Journal of Physiology* **481**, 689–708.

CIVAN, M. M. & PODOLSKY, R. J. (1966). Contraction kinetics of striated muscle fibres following quick changes in load. *Journal of Physiology* **184**, 511–534.

CLEWORTH, D. R. & EDMAN, K. A. P. (1972). Changes in sarcomere length during isometric tension development in frog skeletal muscle. *Journal of Physiology* **224**, 1–17.

CURTIN, N. A. & EDMAN, K. A. P. (1994). Force–velocity relation for frog muscle fibres: effects of moderate fatigue and of intracellular acidification. *Journal of Physiology* **475**, 483–494.

EDMAN, K. A. P. (1979). The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *Journal of Physiology* **291**, 143–159.

EDMAN, K. A. P. (1988). Double-hyperbolic force–velocity relation in frog muscle fibres. *Journal of Physiology* **404**, 301–321.

EDMAN, K. A. P., ELZINGA, G. & NOBLE, M. I. M. (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *Journal of Physiology* **281**, 139–155.

EDMAN, K. A. P., ELZINGA, G. & NOBLE, M. I. M. (1981). Critical sarcomere extension required to recruit a decaying component of extra force during stretch in tetanic contractions of frog skeletal muscle fibers. *Journal of General Physiology* **78**, 365–382.

EDMAN, K. A. P., ELZINGA, G. & NOBLE, M. I. M. (1982). Residual force enhancement after stretch of contracting frog single muscle fibers. *Journal of General Physiology* **80**, 769–784.

EDMAN, K. A. P., ELZINGA, G. & NOBLE, M. I. M. (1984). Stretch of contracting muscle fibres: Evidence for regularly spaced active sites along the filaments and enhanced mechanical performance. In *Contractile Mechanisms in Muscle*, ed. POLLACK, G. H. & SUGI, H., pp. 739–751. Plenum Publishing Corporation, New York.

EDMAN, K. A. P. & LOU, F. (1990). Changes in force and stiffness induced by fatigue and intracellular acidification in frog muscle fibres. *Journal of Physiology* **424**, 133–149.

EDMAN, K. A. P. & REGGIANI, C. (1984). Redistribution of sarcomere length during isometric contraction of frog muscle fibres and its relation to tension creep. *Journal of Physiology* **351**, 169–198.

FENN, W. O. (1924). The relation between the work performed and the energy liberated in muscular contraction. *Journal of Physiology* **58**, 373–395.

FLITNEY, F. W. & HIRST, D. G. (1978). Cross-bridge detachment and sarcomere 'give' during stretch of active frog's muscle. *Journal of Physiology* **276**, 449–465.

FORD, L. E., HUXLEY, A. F. & SIMMONS, R. M. (1977). Tension responses to sudden length change in stimulated frog muscle fibres near slack length. *Journal of Physiology* **269**, 441–515.

GARZIA-NUNZI, M. & FRANZINI-ARMSTRONG, C. (1980). Trabecular network in adult skeletal muscle. *Journal of Ultrastructure Research* **73**, 21–26.

HILL, A. V. & HOWARTH, J. V. (1959). The reversal of chemical reactions in contracting muscle during an applied stretch. *Proceedings of the Royal Society* B **151**, 169–193.

HILL, L. (1977). A-band length, striation spacing and tension change on stretch of active muscle. *Journal of Physiology* **266**, 677–685.

HUXLEY, A. F. (1980). Reflections on muscle. The Sherrington Lectures XIV. Liverpool University Press, Liverpool, UK.

JEWELL, B. R. & WILKIE, D. R. (1958). An analysis of the mechanical components in frog's striated muscle. *Journal of Physiology* **143**, 515–540.

JULIAN, F. J. & MORGAN, D. L. (1979). The effect on tension of non-uniform distribution of length changes applied to frog muscle fibres. *Journal of Physiology* **293**, 379–392.

KATZ, B. (1939). The relation between force and speed in muscular contraction. *Journal of Physiology* **96**, 45–64.

LOMBARDI, V. & PIAZZESI, G. (1990). The contractile response during steady lengthening of stimulated frog muscle fibres. *Journal of Physiology* **431**, 141–171.

MÅNSSON, A. (1994). The tension response to stretch of intact skeletal muscle fibres of the frog at varied tonicity of the extracellular medium. *Journal of Muscle Research and Cell Motility* **15**, 145–157.

MARUYAMA, K., MATSUBARA, S., NATORI, R., NONOMURA, Y., KIMURA, S., OHASHI, K., MURAKAMI, F., HANDA, S. & EGUCHI, G. (1977). Connectin, an elastic protein of muscle. Characterization and function. *Journal of Biochemistry* **82**, 317–337.

MASON, P. (1978). Dynamic stiffness and crossbridge action in muscle. *Biophysics of Structure and Mechanism* **4**, 15–25.

MORGAN, D. L. (1990). New insights into the behaviour of muscle during active lengthening. *Biophysical Journal* **57**, 209–221.

MORGAN, D. L. (1994). An explanation for residual increased tension in striated muscle after stretch during contraction. *Experimental Physiology* **79**, 831–838.

NOBLE, M. I. M. (1992). Enhancement of mechanical performance of striated muscle by stretch during contraction. *Experimental Physiology* **77**, 539–552.

PIAZZESI, G., FRANCINI, F., LINARI, M. & LOMBARDI, V. (1992). Tension transients during steady lengthening of tetanized muscle fibres of the frog. *Journal of Physiology* **445**, 659–711.

STIENEN, G. J. M., VERSTEEG, P. G. A., PAPP, Z. & ELZINGA, G. (1991). Mechanical properties of skinned rabbit psoas and soleus muscle fibres during lengthening: effects of phosphate and Ca^{2+}. *Journal of Physiology* **451**, 503–523.

SUGI, H. (1972). Tension changes during and after stretch in frog muscle fibres. *Journal of Physiology* **225**, 237–253.

SUGI, H. & TSUCHIYA, T. (1981). Enhancement of mechanical performance in frog skeletal muscle fibres after quick increases in load. *Journal of Physiology* **319**, 239–252.

SUGI, H. & TSUCHIYA, T. (1988). Stiffness changes during enhancement and deficit of isometric force by slow length changes in frog skeletal muscle fibres. *Journal of Physiology* **407**, 215–229.

TSUCHIYA, T. & EDMAN, K. A. P. (1990). Mechanism of force enhancement after stretch in intact single muscle fibres of the frog. *Acta Physiologica Scandinavica* **140**, 23A.

WANG, K., MCCARTER, R., WRIGHT, J., BEVERLY, J. & RAMIREZ-MITCHELL, R. (1993). Viscoelasticity of the sarcomere matrix of skeletal muscles. The titin–myosin composite filament is a dual-stage molecular spring. *Biophysical Journal* **64**, 1161–1177.

WANG, K. & RAMIREZ-MITCHELL, R. (1983). A network of transverse and longitudinal intermediate filaments is associated with sarcomeres of adult vertebrate skeletal muscle. *Journal of Cell Biology* **96**, 562–570.

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